

METABOLIC SIGNIFICANCE OF THE DIETARY CMP REQUIREMENT IN RELATION TO *ARTEMIA* MORPHOGENESIS

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ABSTRACT

In *Artemia*, the effect of actinomycin D, which inhibits RNA synthesis, and the effect of puromycin, which inhibits protein synthesis, depend on the AMP:CMP ratio of the diet. When RNA synthesis is limited by CMP concentrations deficient in relation to AMP, protein synthesis decreases and the abdominal length of *Artemia* increases. When RNA synthesis is limited by AMP concentrations deficient in relation to CMP, protein synthesis increases and *Artemia* abdominal segments develop supernumerary gonopodes. Induction of supernumerary gonopodes by a reduced AMP:CMP ratio is suppressed by an albumin deficiency and by puromycin. These results suggest that supernumerary gonopodes are induced by a reduced RNA:protein ratio.

INTRODUCTION

The artificial medium proposed by Provasoli and d'Agostino (1969) for the axenic culture of *Artemia* contains a mixture of adenylic acid, guanylic acid, cytidylic acid, uridylic acid, and thymidine. The purine and pyrimidine requirement of *Artemia* can be satisfied by adenylic acid and cytidylic acid; the quantitative adenylic acid requirement depends on the cytidylic acid concentration of the diet and vice-versa. Thymidine is not essential in a medium containing folic acid, provided the cytidylic acid is not limiting in relation to the adenylic acid concentration in the medium (Hernandorena, 1979). The ratio between dietary adenylic acid and cytidylic acid concentrations modifies not only growth and survival rates of larvae but also their morphogenesis. A dietary adenylic-acid deficiency induces supernumerary gonopodes on abdominal segments (Hernandorena, 1970), but not if the medium is lacking pyrimidines (Hernandorena, 1977). The same supernumerary gonopodes are induced by excess cytidylic acid in a medium containing the standard adenylic-acid concentration, but not if the medium contains excess adenylic acid (Hernandorena 1977, 1979). Thus, the morphogenetic action results from a reduced adenylic acid:cytidylic acid ratio.

Hernandorena (1975) examined the metabolic significance in nucleic acid metabolism and protein synthesis of the dietary adenylic acid requirement, using a medium containing a standard pyrimidines supply (cytidylic acid 10 mg %, uridylic acid 10 mg %, thymidine 5 mg %). In these conditions, effects of actinomycin D decrease with increasing adenylic acid concentration up to 140 mg %, and effects of puromycin decrease with increasing adenylic acid concentration up to 100 mg %. The present report is concerned with the metabolic significance of the dietary adenylic acid:cytidylic acid ratio.

MATERIALS AND METHODS

The method developed by Provasoli and d'Agostino (1969) for axenic culture of the *Artemia* Utah strain was used. Dietary purine requirement was met by adenylic acid (AMP) and pyrimidine requirement by cytidylic acid (CMP). Albumin concentration was 20 mg % unless otherwise stated. The antibiotics used were from Nutritional Biochemical Co. Rearing temperature was $25^{\circ} \pm 0.5^{\circ}$, salinity 24‰; 10 ml of medium was used to rear five animals, and 50–100 larvae were reared for each experimental conditions. Growth is estimated by a numerical index defined by Provasoli and d'Agostino (1969). This growth index approximates number of molts and size. For graphic comparisons of the experiments, the growth index for the 14th day of development was reported.

RESULTS

Results are summarized graphically. Figure 1 shows that the effect of actinomycin D, an inhibitor of RNA synthesis, decreased with increasing CMP concentration provided AMP was more than 20 mg %. RNA synthesis was not limited by CMP with a 20 mg % AMP concentration. RNA synthesis was limited by CMP concentrations of 0–40 mg % with a 40 mg % AMP concentration, of 0–60 mg % with a 60 mg % AMP concentration, of 0–100 mg % with a 100 mg % AMP concentration. The CMP concentrations inducing supernumerary gonopodes are exactly those found to exceed the minimum CMP requirement for maximum RNA

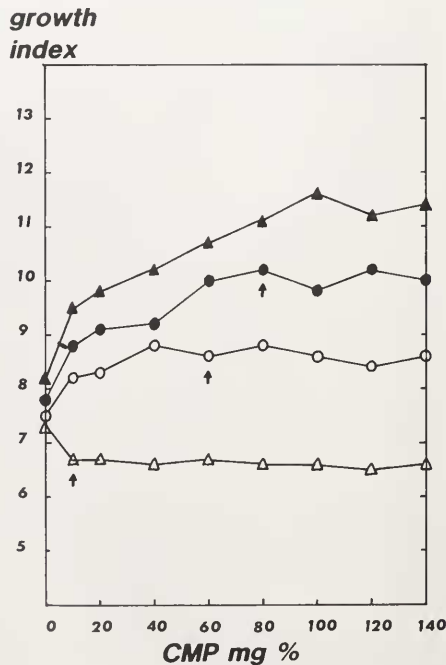


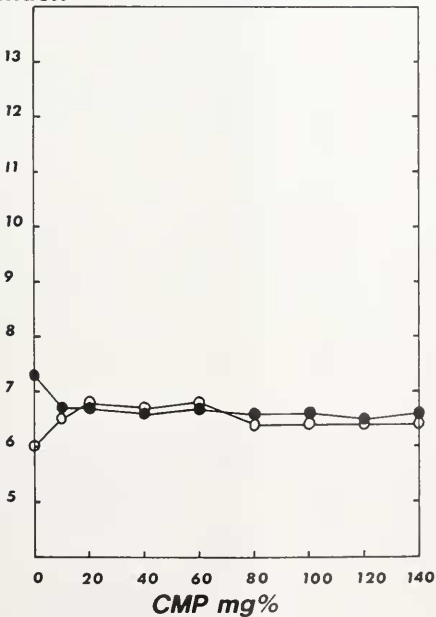
FIGURE 1. Effect on growth of actinomycin D ($2 \mu\text{g} \%$) in relation to the AMP: CMP ratio. Open triangles = AMP: 20 mg %; Open circles = AMP: 40 mg %. Filled circles = AMP: 60 mg %; Filled triangles = AMP: 100 mg %. The arrows indicate minimum CMP concentrations inducing supernumerary gonopodes.

synthesis (arrows on Fig. 1). The threshold increased with increasing AMP concentrations.

The effect of puromycin, an inhibitor of protein synthesis, also depended on the AMP:CMP ratio of the diet (Fig. 2-5). With a 60 mg % AMP concentration, puromycin action decreased with increasing CMP concentration above the concentration found necessary for maximum RNA synthesis. Excess CMP did not induce supernumerary gonopods in the presence of the antibiotic. With apparently the same RNA synthesis, more proteins were synthesized with a reduced AMP:CMP ratio than with a 60:60 ratio (Fig. 4). Similarly, more proteins were synthesized with a reduced ratio than with a 40:40 ratio (Fig. 3). At AMP concentrations of 100 mg %, excess CMP does not induce supernumerary gonopods (Hernandorena, 1979). In this case, effects of both actinomycin D and puromycin decreased with increasing CMP concentrations (Fig. 5). With a 20 mg % AMP concentration, actinomycin D and puromycin were detrimental. Supernumerary gonopods were induced provided the medium was not lacking pyrimidines (Hernandorena, 1977). Lack of pyrimidines, which did not decrease RNA synthesis, decreased protein synthesis (Fig. 2).

According to these results, the supernumerary gonopods were induced by increased protein synthesis. If this is the case, an albumin deficiency or an albumin excess, which both increase the detrimental effect of puromycin (Hernandorena, 1975) should suppress the induction of supernumerary gonopods in larvae reared in a medium containing excess cytidylic acid in relation to the adenylic acid con-

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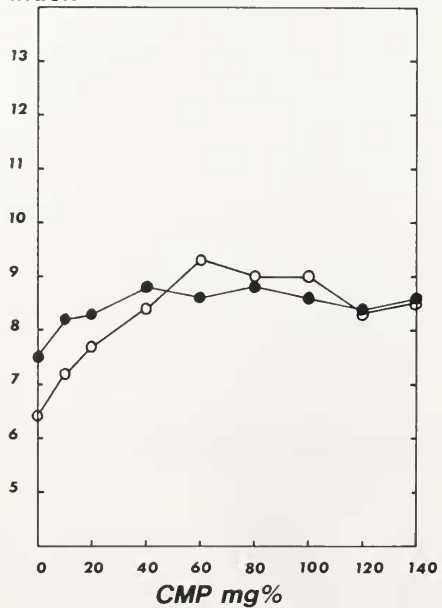
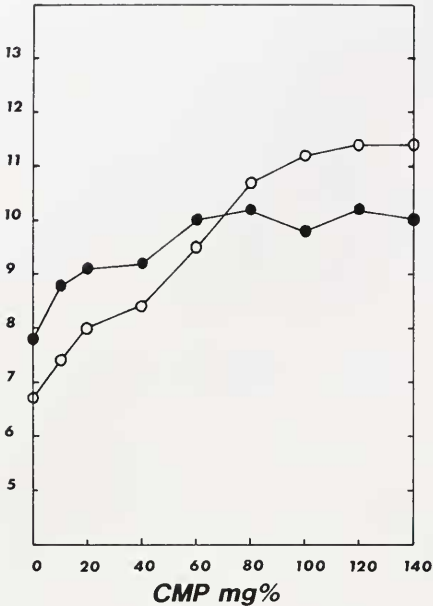


FIGURE 2. (Left.) Effect on growth of actinomycin D (2 μg %) (filled circles) and of puromycin (200 μg %) (open circles) in relation to the AMP:CMP ratio. AMP constant at 20 mg %.

FIGURE 3. (Right.) Effect on growth of actinomycin D (2 μg %) (filled circles) and of puromycin (200 μg %) (open circles) in relation to the AMP:CMP ratio. AMP constant at 40 mg %.

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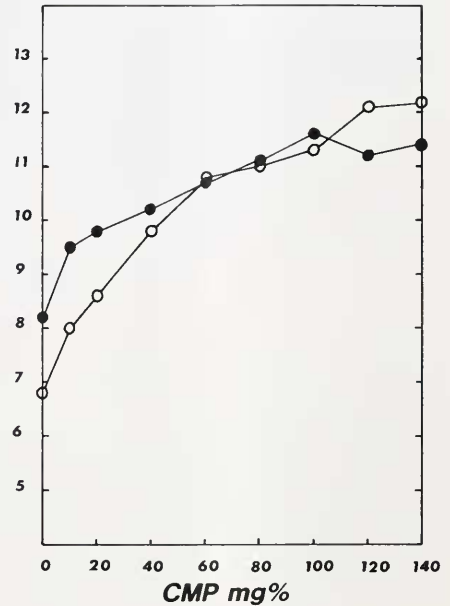


FIGURE 4. (Left.) Effect on growth of actinomycin D ($2 \mu\text{g} \%$) (filled circles) and of puromycin ($200 \mu\text{g} \%$) (open circles) in relation to the AMP:CMP ratio. AMP constant at $60 \text{ mg} \%$.

FIGURE 5. (Right.) Effect on growth of actinomycin D ($2 \mu\text{g} \%$) (filled circles) and of puromycin ($200 \mu\text{g} \%$) (open circles) in relation to the AMP:CMP ratio. AMP constant at $100 \text{ mg} \%$.

centration. As seen in Table I, an albumin deficiency but not an albumin excess suppressed the morphogenetic action of a reduced AMP:CMP ratio.

DISCUSSION

The results obtained by studying the effects of actinomycin D and of puromycin in relation to the AMP:CMP ratio in the diet suggest that the supernumerary gonopodes induced in larvae reared in a medium containing a reduced AMP:CMP ratio result from a reduced RNA:protein ratio.

Artemia, being incapable of *de novo* synthesis of the purine ring (Clegg *et al.*,

TABLE I

Effects of a reduced AMP:CMP ratio in relation to the albumin concentration of the diet.

AMP = $60 \text{ mg} \%$ CMP = $100 \text{ mg} \%$	Growth index 14th day	Larval survival ¹ (percentage)	Morphogenesis of appendages
Albumin $\text{mg} \%$			
5	9.6	90	normal
20	11.0	80	supernumerary gonopodes
60	9.5	60	supernumerary gonopodes

¹ Percentage of larvae reaching growth index 10, independent of their growth rate.

1967; Warner and McClean, 1968) requires a dietary source of purine. *Artemia* can synthesize the pyrimidine ring from the usual precursors (Warner and McClean, 1968) but apparently at a limiting rate under our experimental conditions since growth rate and surviving numbers of larvae increase with increasing CMP dietary supply, provided adenylic acid is not limiting (Hernandorena, 1979). Our present results show that the CMP requirement for RNA synthesis depends on the AMP concentration. Consequently, the metabolic fate of CMP should depend on the AMP concentration. Protein synthesis is known to be an expensive chemical work in terms of ATP cost. With a given AMP concentration, excess CMP could contribute to increased energy production for "extra" protein synthesis.

An enzyme that converts CMP to CDP-choline is present in microsomal preparations of *Artemia* larvae (Ewing and Finamore, 1970a). This reaction could explain the increased energy production resulting from excess CMP. CDP-choline is used for phosphatidyl choline (lecithin) synthesis, and the rate of CDP-choline production from phosphatidyl choline is about twice the rate of synthesis of phosphatidyl choline from CDP-choline. The reversibility of the choline phosphotransferase reaction may be unique to *Artemia*. "This could be a means of energy conservation since the amount of energy invested in the activation of the choline moiety for synthesis of phosphatidyl choline could be salvaged as CDP-choline during degradation and reorganization of membrane structures" (Ewing and Finamore, 1970b).

Present and previous results strengthen this interpretation of the metabolic significance of a reduced AMP:CMP ratio. Induction of supernumerary gonopodes is associated with a reduced abdominal length (Hernandorena, 1972, 1975). Before my 1977 publication, the standard pyrimidine supply was used and the AMP:CMP ratio was increased by increasing AMP concentration. The abdominal length of *Artemia* increases with increasing AMP concentration in the diet. Increasing salinity has the same morphogenetic effect on larvae reared with a standard AMP concentration (Hernandorena, 1975). The effect of puromycin increases with increasing salinity both in nauplii (Ewing *et al.*, 1972) and during larval growth (Hernandorena, 1975). Conditions which limit the rate of protein synthesis, *i.e.*, albumin deficiency or increased salinity, suppress the induction of supernumerary gonopodes on larvae reared in an adenylic acid-deficient medium containing the standard pyrimidine supply (Hernandorena, 1974).

Present results showed that with a high AMP:CMP ratio, protein synthesis decreased, and that an albumin deficiency also suppressed the induction of supernumerary gonopodes on larvae reared in a cytidylic-acid-rich medium. Thus a reduced AMP:CMP ratio induces a morphogenetic action provided protein synthesis is not limited by an albumin deficiency. Albumin deficiency and excess both reduced growth rate; and, as already mentioned (Hernandorena 1975, 1977), the induction of supernumerary gonopodes is independent of larval growth rate but associated with a reduced survival. Albumin deficiency, which suppressed the morphological action, also increased the percentage of surviving larvae, while an albumin excess did not do so (Table I).

Increased protein synthesis resulting from excess CMP in relation to AMP concentration is further supported by the effect of dietary lecithin. Dietary lecithin raises the threshold of AMP concentrations inducing supernumerary gonopodes from 20 mg % to 40 mg % in larvae reared in a medium containing the standard pyrimidines supply (Hernandorena, 1976).

If the above interpretation is correct, a problem remains: How can quantitative differences in the rate of protein synthesis modify the competence of abdominal

segments to develop supernumerary gonopodes and modify the abdominal length of *Artemia*? During *Acetabularia mediterranea* morphogenesis, inhibition of protein synthesis by puromycin induces a loss of morphogenetic capacities (Brachet *et al.*, 1964). In *Artemia*, puromycin suppressed the induction of supernumerary gonopodes. Comparative nutritional data suggest that we are dealing with fundamental processes. In *Drosophila*, the same AMP:CMP ratio is involved in tumorigenesis (Sang and Burnet, 1963a) and in the expression of the eyeless phenotype (Sang and Burnet 1963b). Interestingly, the expression of these same mutant genes is altered by juvenile hormone mimics (Bryant and Sang, 1968). Dietary lecithin is also implicated in tumorigenesis (Sang and Burnet, 1967). Tumor formation in *Musca domestica* is related directly to the animal's ability to synthesize proteins (Bodnaryk, 1968). In *Drosophila* abdomens, the penetrance and expressivity of the abnormal mutation (A^{53g}) can be reduced by adding inhibitors of protein synthesis, RNA synthesis, and oxidative phosphorylation to the diet. The metabolic inhibitors that reduce the expressivity of the mutation also lower the protein concentration of the mutant flies. Adding adenosine to a cytidine-rich, adenosine-deficient diet increases both penetrance and expressivity to a normal level. The details on the effect of the abnormal abdomen genotype remain unknown (Hillman *et al.*, 1973).

In *Artemia*, the effect of RNA-synthesis limitation on protein synthesis and consequently on morphogenesis depends on the metabolic cause of the limitation: AMP deficiency and CMP deficiency have opposite effects. Further biochemical and nutritional investigations are needed.

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